PATENT

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9/24/01 — Stephen J. Rosenman
Date — Stephen J. Rosenman, Ph.D.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants	:	Anne N. Murphy et al.
Application No.	:	09/709,785
Filed	:	November 3, 2000
For	:	COMPOSITIONS AND METHODS FOR DETERMINING INTERACTIONS OF MITOCHONDRIAL COMPONENTS, AND FOR IDENTIFYING AGENTS THAT ALTER SUCH INTERACTIONS
Examiner	:	Arun Chakrabarti
Art Unit	:	1655
Docket No.	:	660088.433C1
Date	:	September 24, 2001

Commissioner for Patents
Washington, DC 20231

AMENDMENT AND RESPONSE TO RESTRICTION REQUIREMENT

Commissioner for Patents:

In response to the Restriction Requirement dated May 22, 2001, please extend the period of time for response three months, to expire on Monday, September 24, 2001. Enclosed are a Petition for an Extension of Time and the requisite fee. Please amend the application as follows.

In the claims:

Please cancel claims 1-91 without prejudice to the filing of any divisional, continuation, or continuation-in-part application.

Please add the following new claims 92-139:

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92. (New) A method of identifying an agent that alters binding of an adenine nucleotide translocator polypeptide to a cyclophilin polypeptide, comprising:

(a) contacting, in the absence and presence of a candidate agent, (i) a first isolated recombinant polypeptide comprising a cyclophilin polypeptide or variant thereof with (ii) a sample comprising a second isolated recombinant polypeptide that comprises a recombinant human adenine nucleotide translocator polypeptide or variant thereof, under conditions and for a time sufficient to permit the cyclophilin polypeptide, the adenine nucleotide translocator polypeptide and the candidate agent to interact; and

(b) comparing a level of binding of the first isolated recombinant polypeptide to the second isolated recombinant polypeptide in the absence of the candidate agent to the level of binding of the first isolated recombinant polypeptide to the second isolated recombinant polypeptide in the presence of the candidate agent, wherein a decreased level of binding in the presence of the agent indicates an agent that inhibits binding of an adenine nucleotide translocator polypeptide to a cyclophilin polypeptide and wherein an increased level of binding in the presence of the agent indicates an agent that enhances binding of an adenine nucleotide translocator polypeptide to a cyclophilin polypeptide, and therefrom identifying an agent that alters binding of an adenine nucleotide translocator polypeptide to a cyclophilin polypeptide.

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93. (New) The method of claim 92 wherein at least one of the first and second isolated recombinant polypeptides is a fusion polypeptide.

94. (New) The method of claim 92 wherein the first isolated recombinant polypeptide comprises a human cyclophilin D polypeptide that is fused to an additional polypeptide, wherein the additional polypeptide is other than glutathione-S-transferase,

95. (New) The method of claim 92 wherein the cyclophilin polypeptide is selected from the group consisting of human cyclophilin A, human cyclophilin B, human cyclophilin C and human Cyp-60.

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96. (New) The method of claim 92 wherein the first isolated recombinant polypeptide comprises a cyclophilin polypeptide fused to an additional polypeptide that is

selected from the group consisting of polyhistidine, polylysine, a haemagglutinin epitope tag, an XPRESS™ epitope tag, a FLAG® epitope tag, a Myc epitope polypeptide, a FLASH peptide, an immunoglobulin constant region polypeptide, streptavidin, a green fluorescent protein polypeptide, an aequorin polypeptide, a glutathione-S-transferase polypeptide and a *Staphylococcus aureus* protein A polypeptide.

97. (New) The method of claim 92 wherein the first isolated recombinant polypeptide is detectably labeled with a linked reporter group.

98. (New) The method of claim 92 wherein the first isolated recombinant polypeptide comprises a cyclophilin polypeptide fused to an additional polypeptide that is polylysine and the second isolated recombinant polypeptide comprises a recombinant human adenine nucleotide translocator polypeptide fused to an XPRESS™ epitope tag.

99. (New) The method of claim 98 wherein the first isolated recombinant polypeptide is detectably labeled with a linked reporter group.

100. (New) The method of either claim 97 or claim 99 wherein the linked reporter group is selected from the group consisting of a radioactive reporter group, a dye, an enzyme, a ligand, a receptor, a protease recognition sequence, a luminescent reporter group and a fluorescent reporter group.

101. (New) The method of claim 92 wherein the sample which comprises the second isolated recombinant polypeptide comprises at least one isolated mitochondrion.

102. (New) The method of claim 92 wherein the sample which comprises the second isolated recombinant polypeptide comprises at least one submitochondrial particle.

103. (New) The method of claim 92 wherein the sample which comprises the second isolated recombinant polypeptide is immobilized on a solid support.

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104. (New) The method of claim 92 wherein the second isolated recombinant polypeptide comprises a human adenine nucleotide translocator polypeptide or variant thereof that is fused to an additional polypeptide selected from the group consisting of polyhistidine, polylysine, a haemagglutinin epitope tag, an XPRESS™ epitope tag, a FLAG® epitope tag, a Myc epitope polypeptide, a FLASH peptide, an immunoglobulin constant region polypeptide, streptavidin, a green fluorescent protein polypeptide, an aequorin polypeptide, a glutathione-S-transferase polypeptide and a *Staphylococcus aureus* protein A polypeptide.

105. (New) The method of claim 92 wherein the step of comparing binding levels comprises detection of a detection reagent that specifically binds to at least one of the polypeptides selected from the group consisting of the first isolated recombinant polypeptide and the second isolated recombinant polypeptide.

106. (New) The method of claim 105 wherein the detection reagent is an antibody.

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107. (New) The method of claim 106 wherein the second isolated recombinant polypeptide comprises a human adenine nucleotide translocator polypeptide or variant thereof that is fused to a polypeptide selected from the group consisting of an XPRESS™ epitope tag and a FLAG® epitope tag, and wherein the antibody specifically binds to at least one polypeptide selected from the group consisting of the human adenine nucleotide translocator polypeptide, the XPRESS™ epitope tag and the FLAG® epitope tag.

108. (New) The method of claim 92 wherein the first isolated recombinant polypeptide comprises human cyclophilin D and wherein the sample which comprises the second isolated recombinant polypeptide comprises at least one submitochondrial particle isolated from a *T. ni* cell that expresses a recombinant human adenine nucleotide translocator-3 polypeptide fused to an XPRESS™ epitope tag.

109. (New) A nucleic acid expression construct comprising an expression control sequence operably linked to a polynucleotide encoding a mitochondrial permeability transition pore component polypeptide or a variant thereof fused to an additional polypeptide or a

variant thereof, wherein the mitochondrial permeability transition pore component is an adenine nucleotide translocator selected from the group consisting of human ANT1, human ANT2 and human ANT3, and wherein the expression control sequence is selected from the group consisting of a regulated promoter and an externally regulated promoter.

110. (New) The method of claim 109 where the externally regulated promoter is a tightly regulated promoter.

111. (New) The expression construct of claim 109 wherein the fused additional polypeptide is selected from the group consisting of polyhistidine, polylysine, a haemagglutinin epitope tag, a FLAG® epitope tag and an XPRESS™ epitope tag.

112. (New) The expression construct of claim 109 wherein the fused additional polypeptide is selected from the group consisting of a Myc polypeptide, a FLASH peptide, an immunoglobulin constant region polypeptide, streptavidin, a green fluorescent protein polypeptide, an aequorin polypeptide, streptavidin, a glutathione-S-transferase polypeptide and a *Staphylococcus aureus* protein A polypeptide.

113. (New) A nucleic acid expression construct comprising an expression control sequence operably linked to a polynucleotide encoding a cyclophilin polypeptide or a variant thereof fused to an additional polypeptide or a variant thereof, wherein the cyclophilin is cyclophilin D and wherein the additional polypeptide is other than glutathione-S-transferase.

114. (New) The expression construct of claim 113, wherein the fused additional polypeptide is selected from the group consisting of polyhistidine, polylysine, a haemagglutinin epitope tag, a FLAG® epitope tag and an XPRESS™ epitope tag.

115. (New) The expression construct of claim 113, wherein the fused additional polypeptide is selected from the group consisting of a Myc polypeptide, a FLASH peptide, an immunoglobulin constant region polypeptide, a green fluorescent protein polypeptide, an aequorin polypeptide, streptavidin and a *Staphylococcus aureus* protein A polypeptide.

116. (New) A nucleic acid expression construct comprising an expression control sequence operably linked to a polynucleotide encoding a cyclophilin polypeptide or a variant thereof fused to an additional polypeptide or a variant thereof, wherein the cyclophilin polypeptide is selected from the group consisting of human cyclophilin A, cyclophilin B, human cyclophilin C and human Cyp-60.

117. (New) The expression construct of claim 116 wherein the fused additional polypeptide is selected from the group consisting of polyhistidine, polylysine, a haemagglutinin epitope tag, an XPRESS™ epitope tag, a FLAG® epitope tag, a Myc epitope polypeptide, a FLASH peptide, an immunoglobulin constant region polypeptide, streptavidin, a green fluorescent protein polypeptide, an aequorin polypeptide, a glutathione-S-transferase polypeptide and a *Staphylococcus aureus* protein A polypeptide.

118. (New) An expression construct according to any one of claims 109, 112 or 116 wherein the construct comprises a vector selected from the group consisting of a plasmid, a cosmid, a shuttle vector, a viral vector and a vector comprising a chromosomal origin of replication.

119. (New) An expression construct according to claim 118 wherein the vector is selected from the group consisting of pBAD-His, pEYFP-C1, and pECFP-N1.

120. (New) An isolated polypeptide comprising a mitochondrial permeability transition pore component polypeptide or a variant thereof fused to an additional polypeptide or a derivative thereof, wherein the mitochondrial permeability transition pore component is an adenine nucleotide translocator selected from the group consisting of human ANT1, human ANT2 and human ANT3.

121. (New) The polypeptide of claim 120 wherein the fused additional polypeptide is selected from the group consisting of polyhistidine, polylysine, a haemagglutinin epitope tag, a FLAG® epitope tag and an XPRESS™ epitope tag.

122. (New) The polypeptide of claim 120 wherein the fused additional polypeptide is selected from the group consisting of a Myc epitope polypeptide, a FLASH peptide, an immunoglobulin constant region polypeptide, streptavidin, a green fluorescent protein polypeptide, an aequorin polypeptide, a glutathione-S-transferase polypeptide and a *Staphylococcus aureus* protein A polypeptide.

123. (New) An isolated polypeptide comprising a mitochondrial permeability transition pore component polypeptide or a variant thereof fused to an additional polypeptide or a derivative thereof, wherein the mitochondrial permeability transition pore component is selected from the group consisting of porin, hexokinase, creatine kinase, PRAX, CAML and the peripheral benzodiazepine receptor.

124. (New) The polypeptide of claim 123 wherein the fused additional polypeptide is selected from the group consisting of polyhistidine, polylysine, a haemagglutinin epitope tag, a FLAG® epitope tag and an XPRESS™ epitope tag.

125. (New) The polypeptide of claim 123 wherein the fused additional polypeptide is selected from the group consisting of a Myc epitope polypeptide, a FLASH peptide, an immunoglobulin constant region polypeptide, streptavidin, a green fluorescent protein polypeptide, an aequorin polypeptide, a glutathione-S-transferase polypeptide and a *Staphylococcus aureus* protein A polypeptide.

126. (New) An isolated polypeptide comprising a cyclophilin or a variant thereof fused to an additional polypeptide or a variant thereof, wherein the cyclophilin is cyclophilin D and wherein the additional polypeptide is selected from the group consisting of polyhistidine, polylysine, a haemagglutinin epitope tag, a FLAG® epitope tag and an XPRESS™ epitope tag.

127. (New) An isolated polypeptide comprising a cyclophilin or a variant thereof fused to an additional polypeptide or a variant thereof, wherein the cyclophilin is selected from the group consisting of human cyclophilin A, human cyclophilin B, human cyclophilin C and human Cyp-60 .

128. (New) The polypeptide of claim 127, wherein the fused additional polypeptide is selected from the group consisting of polyhistidine, polylysine, a haemagglutinin epitope tag, a FLAG® epitope tag and an XPRESS™ epitope tag.

129. (New) The polypeptide of claim 127, wherein the fused additional polypeptide is selected from the group consisting of a Myc epitope polypeptide, a FLASH peptide, an immunoglobulin constant region polypeptide, streptavidin, a green fluorescent protein polypeptide, an aequorin polypeptide, a glutathione-S-transferase polypeptide and a *Staphylococcus aureus* protein A polypeptide..

130. (New) A polypeptide according to any one of claims 122, 125 and 129 wherein the green fluorescent protein is selected from the group consisting of blue-shifted GFP, cyan-shifted GFP, red-shifted GFP and yellow-shifted GFP.

131. (New) A host cell for identifying agents that alter mitochondrial permeability transition, comprising at least one mitochondrion and a nucleic acid expression construct according to any one of claims 109-118.

132. (New) A host cell according to claim 131 wherein the host cell is a prokaryotic cell.

133. (New) A host cell according to claim 131 wherein the host cell is a eukaryotic cell.

134. (New) A host cell according to claim 133 wherein the eukaryotic cell is derived from a cell line that is selected from the group consisting of 293, COS-7, Sf9, CHO, Hep-2, MDCK, SH-SY5Y and Jurkat.

135. (New) A host cell according to claim 133 wherein the eukaryotic cell is a *T. ni* cell.

136. (New) A host cell according to claim 131 wherein the recombinant expression construct is extrachromosomal.

137. (New) A host cell according to claim 131 wherein the nucleic acid expression construct is integrated into a host cell chromosome.

138. (New) A host cell according to claim 137 wherein the host cell chromosome is a mitochondrial chromosome.

139. A method for preparing a cyclophilin polypeptide fused to an additional polypeptide, comprising the steps of:

(a) culturing, under conditions that permit expression of a fusion protein, a host cell comprising a nucleic acid expression construct that is selected from the group consisting of (i) a nucleic acid expression construct comprising an expression control sequence operably linked to a polynucleotide encoding a cyclophilin polypeptide or a variant thereof fused to an additional polypeptide or a variant thereof, wherein the cyclophilin is cyclophilin D and wherein the additional polypeptide is other than glutathione-S-transferase, and (ii) a nucleic acid expression construct comprising an expression control sequence operably linked to a polynucleotide encoding a cyclophilin polypeptide or a variant thereof fused to an additional polypeptide or a variant thereof, wherein the cyclophilin polypeptide is selected from the group consisting of human cyclophilin A, cyclophilin B, human cyclophilin C and human Cyp-60

encodes a fusion protein comprising a cyclophilin polypeptide or a derivative thereof fused to an energy transfer molecule polypeptide or a derivative thereof; and

(b) recovering fusion protein from the culture.

REMARKS

Claims 1-91 are pending in the present application and all are canceled without prejudice by the amendment submitted herewith. New claims 92-139 have been added by the present amendment to define embodiments of the invention that applicants wish to have examined at this time. Support for this amendment may be found in the specification, for example, at page 49, lines 1-22; at page 50, line 7 through page 51, line 12; and in the Examples (*e.g.*, at pages 68-96, 100-123 and 134-140). No new subject matter has been added to the application.

Therefore, applicants believe the application is in condition for allowance. Favorable consideration of the claims and a Notice of Allowance are earnestly solicited.

Respectfully submitted,

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